Neutrophil gelatinase-associated lipocalin in dehydrated horses: A prospective case-control study

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Abstract

Background

Hypovolemia caused by dehydration could eventually lead to acute renal injury. An early marker for detection of renal injury is still needed in equine medicine. Besides biomarkers which reflect glomerular filtration rate, markers indicating damages in renal tubules might be beneficial. The aim of the study is 1) to estimate both serum and urinary Neutrophil gelatinase-associated lipocalin (sNGAL and uNGAL) concentrations in horses with different severity of dehydration, 2) to analyze the correlations between sNGAL, uNGAL and the traditional renal markers, and 3) to determine if inflammation has an impact on sNGAL, uNGAL concentrations.

Methods

Serum NGAL, urinary NGAL, creatinine and urea concentrations were measured in 38 horses with dehydration and 4 healthy control horses four times totally until 48 hours after admission. Horses were divided into different groups according to the severity of dehydration and with or without systemic inflammatory response syndrome. The Kruskal–Wallis test was used to analyze the differences of sNGAL and uNGAL concentrations among the three dehydration groups and the control group. Linear mixed regression models with repeated measurement were applied to assess the association between NGAL at four time points and four groups independently.

Results

Significant differences of sNGAL ($P = 0.02$) and uNGAL ($P = 0.01$) at T_0 were found between the control and all three dehydration groups. sNGAL and uNGAL both had correlations with serum creatinine and urea concentrations. A significant difference of sNGAL at T_0 has been found between dehydrated horses with and without SIRS ($P < 0.001$), while there was no significant difference of uNGAL between these groups ($P = 0.08$).

Conclusions

Moderate correlations were observed between sNGAL, uNGAL and serum creatinine and urea concentrations. Significant differences of sNGAL and uNGAL between dehydration groups and healthy controls were observed. Neither were associated with the short-term prognosis. Inflammation might affect the interpretation of sNGAL in horses.

Background

Acute kidney injury (AKI) is defined as a rapid decrease in renal function and direct injury to the kidneys, regardless of the underlying diseases [1]. Acute kidney injury is one of the critical factors associated with survival rate, mortality and length of hospitalization of the patients in both humans and dogs [2, 3].

The prevalence of AKI in horses was observed to be around 14.8% in hospitalized horses [4]. Another study indicated that horses with persistent renal insufficiency after 72 h were three times more likely to die or be euthanized than horses with resolved azotemia [5]. An early detection of renal injury would benefit the equine patient, but is still difficult with current diagnostics. Assessment of the glomerular filtration rate (GFR) is known to be the best way to detect early renal dysfunction, however, the challenging technique and equipment required make it impractical for routine use [6]. Although the serum creatinine concentration reflects the GFR quite well, its increase is delayed until a 75% loss of renal function and is influenced by many extrarenal factors, such as body mass, age and breeds [7, 8]. Blood urea nitrogen (BUN) has no direct linear correlation with GFR and is also affected by factors such as diet and liver function [9]. The last publication from the same research group studied the usage of symmetric dimethylarginine (SDMA) in horse, which is also a novel renal biomarker [10]. In our study, the serum SDMA concentration correlated with creatinine and differences in SDMA at admission was detected among dehydration levels, but the distribution of SDMA in the 3 groups overlapped easily with each other. Further research is still needed to identify the role of SDMA in equine renal disease.

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein originally detected in activated neutrophils. It has been shown that NGAL is also produced in the renal tubular epithelia in response to injury. Different forms of NGAL can be measured in serum, plasma and urine and its levels correlate with the severity of the renal impairment [11, 12]. Serum and plasma NGAL increased 24–72 h earlier than creatinine in humans and indicated the acute renal damage in one study [13–16]. Urinary NGAL also showed promising results and was elevated as early as 2 h after ischemic renal damage [17–19].

Based on the origin of NGAL, several studies which discussed its ability to differentiate inflammatory and non-inflammatory disease in equine patients were published [20–22]. However, NGAL's diagnostic value of renal diseases in equine medicine has not yet been widely researched. A new study has found significant differences in the concentration of NGAL in serum and urine between healthy and AKI-affected horses [23].

The aim of our study is to evaluate the serum and urinary NGAL concentration in clinically dehydrated horses which had different levels of dehydration and were prone to develop AKI. It was hypothesized that: 1) both serum and urinary NGAL concentrations would correlate with the severity of dehydration; 2) NGAL would associate with the short-term mortality of the patients; and 3) NGAL would show earlier detection of AKI than traditional biomarker creatinine and BUN.

Results

Study population

A total of 57 dehydrated horses that met the criteria were enrolled in the study group and 4 healthy horses were included in the control group. Sixteen horses were excluded due to a lack of obvious laboratory changes which made grading of the accurate dehydration status impossible. Three horses were excluded because of missing blood samples at the most important T_0. The final study group contained 38 horses.

Eleven horses were assigned to the mildly dehydrated group, 16 to the moderately dehydrated group and 11 that were in hypovolemic shock at admission were in the severely dehydrated group. Only one horse in the moderately dehydrated group developed AKI during hospitalization.

Fourteen horses fitted the criteria of SIRS above score 2: 3 of them were divided into the mildly dehydrated group, while 4 were in the moderately dehydrated group and 7 in the severely dehydrated group.

Of the dehydrated horses, 29 were presented due to gastrointestinal diseases, 2 because of intoxication, 1 with neoplasia and 6 had miscellaneous diseases (lameness, respiratory problems and hemoabdomen). Horses were treated according to their primary disease. A total of 39.5% horses (15/38) received Gentamicin (6.6 mg/kg BW, once daily, IV) during the study period, while 78.9% (30/38) had non-steroidal anti-inflammatory drugs (NSAIDs) at least once. The time points of the medication depended on the course of the disease and were, therefore, inconsistent. Regarding NSAIDs, either Flunixin meglumin 1.1 mg/kg once-twice daily or Phenylbutazone 2.2 mg/kg twice daily were used.

Seventeen horses survived to discharge, while 21 horses died or were euthanized during the hospitalization period. Two horses were euthanized due to financial constraints and were excluded from the final statistical analysis.

The sNGAL, uNGAL, creatinine and BUN in groups

Results of laboratory parameters measured at T_0 in dehydrated and control horses are shown in Table 1. Median concentrations of sNGAL, uNGAL and BUN were lowest in the control group and increased with the severity of dehydration. This could not be shown for creatinine.

Significant differences of these parameters at T_0 were found between the control and all three dehydrated groups (sNGAL: $P = 0.02$; Fig. 1/ uNGAL: $P = 0.01$; Fig. 2/ Creatinine: $P<0.001$/ BUN: $P = 0.02$). The difference in sNGAL and uNGAL was largest between the healthy controls and severely dehydrated horses (sNGAL: $P = 0.002$; uNGAL: $P = 0.02$).

There was no association between concentrations of sNGAL and uNGAL at T_0 and the short-term prognosis within 48h ($P = 0.1$ and $P = 0.8$).

There was a moderate correlation between sNGAL and uNGAL at T_0 (R = 0.580, $P<0.001$). The sNGAL also had weak correlations with creatinine and BUN at T_0 (R = 0.271, $P = 0.01$; R = 0.281, $P = 0.009$, respectively). Similarly, weak correlations between uNGAL and creatinine, and uNGAL and BUN were

found at admission (R = 0.308, P = 0.02; R = 0.382, P = 0.004, repsectively). Correlations were only found between sNGAL and uNGAL (R = 0.619, P = 0.001; R = 0.818, P<0.001; R = 0.511, P = 0.04, respectively), but not among other parameters from T_{12} to T_{48}.

A significant difference of sNGAL at T_0 has been found between dehydrated horses with and without SIRS (P<0.001), while there was no significant difference of uNGAL between the same groups (P =0.08).

According to the linear mixed regression model, neither sNGAL nor uNGAL showed any significant differences between time point and groups. The interaction between the groups and time points showed no significance. In conclusion, sNGAL and uNGAL differentiated between healthy and dehydration groups only at T_0. The estimated marginal means of sNGAL, uNGAL, serum creatinine and BUN concentrations in groups within 48 h are shown in Fig. 3 to 6. Urine of the control horses was only sampled at T_0 and no continuous urine samples from TP_{12} to TP_{48} were successfully taken. Therefore, the results of uNGAL in the control group could not be compared. The lowest mean concentrations of sNGAL throughout 48 h were seen in the control group, while the concentrations in the three dehydrated groups overlapped with each other easily.

sNGAL was associated significantly with the usage of Gentamicin (P =0.01, 0.009, 0.005 and 0.03) from T_0 to T_{48}, while uNGAL was associated with Gentamicin only at T_0 (P = 0.01). Similar results have been observed for groups with and without the application of NSIADs: Except for T_{48}, sNGAL differed significantly between these two groups until T_{24} (P = 0.02, 0.007 and 0.008, respectively), while uNGAL only showed a significant difference at T_0 (P = 0.01). On the other hand, serum creatinine and BUN concentrations associated neither with Gentamicin nor with NSIAD usage during the whole study period.

Table 1 Concentrations of neutrophil gelatinase associated lipocalin, serum creatinine and urea concentrations at time-point 0 in dehydration and healthy control groups

Biomarkers/ Unit	Groups	N	Minimum	Maximum	Median	IQR	P value[a]
sNGAL (ng/mL)	Control	4	9.30	19.60	18.75	15.8-19.6	0.002
	Mild dehydration	11	19.2	308.0	43.8	26.5-104.2	
	Moderate dehydration	16	12.2	5104.0	56.95	29.9-123.8	
	Severe dehydration	11	20.4	1125.4	270.4	119.0-471.6	
	Survivor	17	13.6	1125.4	59.6	23.9-127.2	0.1
	Non-survivor	19	12.2	5104.4	119.60	45.8-296.6	
uNGAL (ng/mL)	Control	4	3.70	10.0	4.5	3.8-6.4	0.01
	Mild dehydration	9	4.5	2037.3	54.4	15.4-284.0	
	Moderate dehydration	10	8	161785.7	183.85	21.1-1064.5	
	Severe dehydration	5	43.3	21135.7	229.0	121.4-409.1	
	Survivor	13	4.5	21135.7	121.4	18.3-409.1	0.8
	Non-survivor	9	8.0	161785.7	284.0	34.5-536.3	
Creatinine (µmol/L)	Control	4	93.28	110.0	95.92	95.3-99.4	<0.001
	Mild dehydration	11	60.6	131.4	83.5	74.7-104.5	
	Moderate dehydration	16	91.6	438.2	149.2	116.5-163.7	
	Severe dehydration	11	96.8	399.5	135.3	116.8-172.6	
	Survivor	17	69.6	344.7	127.8	96.8-154.8	0.2
	Non-survivor	19	60.6	438.20	134.0	111.7-165.1	
BUN	Control	4	4.26	6.03	4.78	4.36-5.38	0.02

(mmol/L)	Mild dehydration	11	3.28	8.90	4.90	4.18-5.65	
	Moderate dehydration	16	3.63	13.87	6.32	5.17-8.07	
	Severe dehydration	11	4.96	10.78	7.21	5.85-8.99	
	Survivor	17	3.28	12.80	5.52	4.26-8.27	0.5
	Non-survivor	19	3.82	13.87	6.17	4.98-8.02	

Abbreviations: BUN, blood urea nitrogen; IQR, interquartile range; N, number of subset; sNGAL, serum neutrophil gelatinase associated lipocalin; uNGAL, urinary neutrophil gelatinase associated lipocalin.

[a] *P* values indicate the differences between groups.

Discussion

This study focused on sNGAL and uNGAL concentrations in dehydrated horses and compared these new markers with the traditional renal markers throughout the first 48 h of hospitalization time.

NGAL is rapidly expressed under various pathological conditions by several cell types, such as immune cells, hepatocytes and renal tubular cells [24]. There are two forms of NGAL identified in humans and animals: a dimeric form which is released by neutrophils and a monomeric form that is secreted by proximal tubular epithelial cells [11, 25, 26]. Several studies showed that both sNGAL and uNGAL can elevate rapidly within 2 h after renal damage and that both plasma and urinary NGAL correlated well with the creatinine concentration [13, 15, 27, 28]. In the present study, significant correlations between sNGAL, uNGAL, creatinine and BUN concentrations at admission were identified. sNGAL and uNGAL correlated with each other throughout the 48 h study period. After T_0, sNGAL and uNGAL did not show a correlation with either creatinine or BUN, which may be due to the different underlying diseases and different treatment regimes in our patients.

A precise cut-off value of NGAL for the usage of detecting AKI has not yet been defined, but sNGAL concentrations were significantly higher in horses with increased creatinine compared to control horses in a previous study [29, 30]. Our study also showed similar results: dehydrated horses had higher mean and median sNGAL than our healthy controls, and what is more, healthy horses in our study all had sNGAL concentrations similar to the control group from the previous publication.

Unlike creatinine, which only increases when the renal damage has already advanced, NGAL tends to show the “active damage” in the kidney and, therefore, is suitable as a real-time indicator of AKI [12, 31, 32]. Except for one horse diagnosed with AKI during its hospitalization, creatinine and BUN concentrations decreased back to the reference range until T_{12} under rehydration therapy. By contrast,

sNGAL and uNGAL remained elevated in most cases and never decreased to the concentrations measured in the healthy control horses (Fig. 3–6). A total of 39.5% (15/38) of the dehydrated horses had continuously increased sNGAL until T_{48} (all of them increased above 10 ng/mL), only three of these 15 horses had serum creatinine concentrations above the reference range at T_0 and only one of these three horses had sustained high creatinine until T_{48}. A total of 37.5% (9/24) had increased uNGAL until T_{48}, 2 of these 9 horses had increased serum creatinine at T_0 but both decreased between T_{12} and T_{24}. The reason for that might be that our patients had sustained minor renal damage even after rehydration therapy that could not be detected by traditional renal markers. Markers for tubular injury, such as NGAL, could be more sensitive than GFR markers, such as creatinine, to detect prerenal AKI because the tubule is more susceptible to hypoperfusion and hypoxic damage than the glomerulus [27, 33, 34]. No significant difference of either sNGAL or uNGAL were identified for time-group interaction and among time points throughout 48 h in this study. There were lots of extrarenal factors affecting NGAL concentrations both in serum and urine because of the various primary disorders and therapy choices of each patient. These influences probably reduced the differences between the three dehydration groups (mild, moderate and severe).

Although two studies in human cardiac patients indicated that plasma NGAL could be a predictive marker of mortality, neither sNGAL nor uNGAL were associated with the survival rate in other studies in dogs [34–36]. Although the one horse diagnosed with AKI in our study was euthanized, neither uNGAL nor sNGAL showed any significant difference between survivors and non-survivors. Therefore, even when NGAL might have shown early renal damage, it did not markedly affect the survival chance in our patient.

Since NGAL also originates from neutrophils, it has been discussed whether inflammation in the body could have an impact on this new marker. One study described that NGAL could also increase in various infectious, malignant disorders and inflammations, even without kidney injury [37]. Research in small animals and humans indicated that there was a significant influence of SIRS on sNGAL, especially, and, therefore, limits its usage in patients with systemic inflammation and possible AKI [11, 35, 38]. This is further complicated by the lack of a uniform definition of the term SIRS in adult horses and the wide variety of existing scoring systems. Most scoring systems in horses resemble those from human medicine that were designed to ensure identification of at-risk patient. This leads to a high sensitivity and poor specificity and one should be careful when applying it to such heterogenous study group as ours. Urinary NGAL is thought to be more specific for diagnosing AKI than sNGAL, nevertheless, in cases with nephritis or pyuria, uNGAL still needs to be interpreted cautiously [24, 36]. A significant difference of sNGAL has been found between dehydrated horses in this study with and without SIRS at admission, while uNGAL showed no difference between these groups.

Gentamicin and NSIADs are known as potential nephrotoxic drugs and might cause transient renal damage through the clinical usage [39, 40]. Although a study in dogs receiving Gentamicin indicated that uNGAL was a sensitive predictor of AKI, our result showed a better distinguishing value of sNGAL than uNGAL: sNGAL associated well with the treatment of Gentamicin in all 15 of our patients which received it at all time points and uNGAL did not, thus, sNGAL might indicate the micro damage of Gentamicin

better than uNGAL [17]. However, we only had urine samples at all time points available in 7/28 horses in our study and, therefore, results after T_0 should be interpreted cautiously. Interestingly, serum creatinine and BUN concentrations did not show any association with the usage of Gentamicin and NSAIDs. It is possible that sNGAL and uNGAL might be able to detect minor renal damage which is caused by nephrotoxic medications earlier than traditional parameters.

The lack of urine samples at all time points and the heterogenous study group were the main limitations of this study. Potentially nephrotoxic medications such as Gentamicin and NSAIDs could not be avoided and were used in different regimes in individual patients. In addition, sNGAL and uNGAL were measured in samples stored at -80 collected and retained for around one year. Validation of the ELISA Kit showed no influence on the NGAL measurement when -80 storage could be provided; the NGAL protein should be stable for at least 11 months under that condition [41]. However, we could not assure whether there were any differences between fresh and frozen samples.

Conclusion

Moderate correlations were observed between sNGAL, uNGAL and traditional renal markers serum creatinine and BUN. sNGAL and uNGAL were significantly higher at admission in all dehydration groups compared to the control group. Neither were associated with the short-term prognosis. They did not decrease significantly after T_{12} in contrast to creatinine and BUN, which might indicate the ability of NGAL to detect minor ongoing renal injury before the patient's full recovery. A SIRS score above 2 at admission was associated with an increased sNGAL. Therefore, uNGAL might be more specific for predicting AKI when there is accompanying systemic inflammation. Furthermore, sNGAL and uNGAL might both be capable of detecting the transient minor renal damage caused by potentially nephrotoxic drugs.

A larger population of equine patients and healthy controls should be examined in the future to define a cut-off value of sNGAL and uNGAL for diagnosing AKI. More prospective studies will be needed to ameliorate the usage of NGAL in horses and assess the influence of sepsis and different types of underlying diseases on NGAL interpretation.

Methods

Study design and study population

This study was a prospective investigation performed on horses admitted to the Equine Clinic: Surgery and Radiology at Freie Universität Berlin between August 2018 and December 2019 with at least 6% dehydration without a primary or history of kidney disease. The study was reported in accordance with ARRIVE guidelines, all patients received appropriate treatments according to their main complaint. Horses that had at least two or more abnormal results of the following criteria on admission were included: heart rate >60 beats/min, packed cell volume(PCV) >40%, total protein concentration (TP) >70 g/L, capillary

refill time >2 s, lactate >0.9 mmol/L and clinical signs indicative of hypovolemic shock, including cold extremities, pale mucous membranes or decreased jugular refill time. The assessment of the grade of dehydration was based on the clinical examination, packed cell volume and TP at admission (Additional file 1) [33]. Foals younger than three months were excluded from the study in order to avoid spurious hypercreatininemia and age related differences [42].

The horses were divided into three groups: those with 1) mild dehydration (6–8%), 2) moderate dehydration (8–10%) and 3) severe dehydration (>10% or if horses were in hypovolemic shock).

Horses with mild dehydration were rehydrated either with infusion therapy (Ringer-lactate or Ringer's solution. B. Braun Melsungen AG, Melsungen, Germany) for at least 24 h, as indicated by PCV and TP, or water by nasogastric tube; horses with moderate or severe dehydration were treated with infusion therapy in all cases. Other treatments were chosen based on the horses' main complaint.

Survivors were defined as horses which survived to discharge; non-survivors were those which died or were euthanized during the hospitalization. Horses which were euthanized due to financial constraints were excluded from the analysis for the prognostic value of NGAL in order to reduce the statistical error.

Acute kidney injury was defined as an increase of serum creatinine concentration≧26.5 μmol/L within 48 h, according to the veterinary AKI staging system [4].

In addition, horses were graded according to a systemic inflammatory response syndrome (SIRS) score by Roy et al., 2017 [43]. SIRS scores from 1 to 4 were made depending on how many abnormal criteria the horses met. Considering that most of our patients were emergency cases, only horses with SIRS above score 2 that fitted two or more of the following criteria in addition to increased lactate concentration (>2.06 mmol/L) and abnormal mucous membranes (colour bright pink, injected, purple, muddy, toxic, red or white) were later statistical analysed: heart rate >52 beats/min, respiratory rate >20 breaths/min, white blood cell count <5 or >12.5 10^9/L, and a body temperature <37 or >38.5°C.

Serum NGAL (sNGAL), urea and creatinine concentrations, and urinary NGAL (uNGAL) were measured in the dehydrated horses and clinically healthy horses throughout 48 h after admission.

Blood sample collection

A total of 10 ml blood was taken from the external jugular vein at time-point 0 (T_0) when the horses arrived at the clinic before infusion therapy began. The same samples were also taken at 12, 24 or 48 ± 2 h (T_{12}, T_{24} and T_{48}, respectively) after admission. The same four-times collection was performed in the control group. Each sample was filled into a serum tube with a clot activator (Sarstedt AG & Co, Nümbrecht, Germany) and centrifuged at 3,800 g for 10 min. Two 1.2 ml samples were frozen at -80 for each time point for the later measurement of sNGAL; one sample was kept at 4 and sent to an external laboratory (SYNLAB. vet GmbH, Berlin, Germany) and analyzed within 24 h. The concentrations of serum creatinine, BUN, glucose, TP, albumin and electrolytes were measured utilizing an automated AU680

clinical chemistry analyser (Beckman Coulter GmbH, Krefeld, Germany). The sNGAL concentrations were measured with a commercial ELISA kit (NGAL Kit no. 49, BioPorto Diagnostics, Denmark) for horses and the measurement was performed according to the manufacturer's instructions. A similar kit has been validated in horses for research purposes [29, 44].

Urine sample collection

Urine samples were taken from mares at T_0 with a urine catheter before or within the first 30 min of infusion therapy; stallions and geldings' urine was collected during surgery, if the horse underwent surgery for its primary disease directly after admission or from the midstream of naturally voided urine in the stable within 30 min of admission. The urine samples at T_{12}, T_{24} and T_{48} were taken in the stable during spontaneous urination after admission. The same collection protocol was also used in the control group. Urine (10 ml) collected in a sterile urine collection tube (Labor- und Medizintechnik Specht GmbH, Eresing, Germany), was centrifuged at 1,500 g for 10 min at 4°C. The supernatant was collected and frozen at -80°C for the later measurement of uNGAL by the same ELISA Kit (NGAL Kit no. 49, BioPorto Diagnostics, Denmark) for horses.

Data analysis

Analysis was performed using IBM SPSS software (IBM Deutschland GmbH, Ehningen, Germany) for Windows, version 25. Creatinine, BUN, sNGAL and uNGAL were analyzed by Shapiro–Wilk tests to check the distribution of parameters. The Kendall Tau b coefficient test was used to determine the correlations between concentrations of sNGAL, creatinine, BUN and uNGAL, respectively, from T_0 to T_{48}. The Kruskal–Wallis test was used to analyze the differences of sNGAL and uNGAL concentrations among the three dehydration groups and the control group. The distribution of serum creatinine concentration and BUN among the four groups at T_0 were also analyzed by the Kruskal–Wallis test. The distribution of sNGAL and uNGAL at T_0 in survivors/ non-survivors groups, and the difference between horses with or without SIRS were analyzed by the Mann–Whitney U test. Linear mixed regression models with repeated measurement were applied to assess the association between NGAL at four time points and four groups independently. Mauchly's test for sphericity was applied and the Huynh-Feldt correction used to determine differences between the time points and interactions between the time point and group. Model diagnostics included visual inspection of residuals for normality. The level of significance was set at 5% for all analyses.

Abbreviations

AKI	Acute kidney injury
BUN	Blood urea nitrogen
GFR	Glomerular filtration rate
NGAL	Neutrophil gelatinase associated lipocalin
NSIADs	Non-steroidal anti-inflammatory drugs
PCV	Packed cell volume
SDMA	Symmetric dimethylarginine
SIRS	Systemic inflammatory response syndrome
TP	Total protein concentration

Declarations

a. Ethics approval and consent to participate

The study was not classified as an animal experiment by the State Office of Berlin (O 0218/19). Owners were informed and gave written consent to involve their horses' data anonymously with contract governing medical treatment of the Equine Clinic of Freie Universitaet Berlin. All methods that conducted in this study were performed in accordance with the relevant guidelines and regulations.

b. Consent for publication

Not applicable.

c. Availability of data and materials

The datasets generated during and analyzed during the current study are not publicly available due to the privacy protection of the participants but are available from the corresponding author on reasonable request.

d. Competing interests

Author Hsiao-Chien, Lo received support for this work from SYNLAB.vet GmbH, Berlin, including part of study design, kits for measurement with the coordination of co-author Judith C. Winter. The author has full access to the study data and take complete responsibility for the integrity of the data and accuracy of data analysis. Other co-authors have declared no competing interests.

e. Funding

The ELISA-Kit (NGAL Kit no. 49, BioPorto Diagnostics, Denmark) for measurement of Neutrophil gelatinase-associated lipocalin (NGAL, object Biomarker in this study) were provided by SYNLAB.vet

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e. Authors' information (optional)

Hsiao-Chien, Lo was the principal author and contributed to study design, data collection, and data analysis, and manuscript preparation. Judith C. Winter contributed to study design, project coordination and revising the content. Merle Roswitha contributed to data analysis and interpretation. Heidrun Gehlen was the senior author and contributed to overall study design, project coordination, data analysis and revising the manuscript.

References

1. Kidney Disease Improving Global Outcomes (KDIGO). Section 2: AKI definition. Kidney Int Suppl. 2012;2:19–36.
2. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol. 2005;16:3365–3370.
3. Thoen ME, Kerl ME. Characterization of acute kidney injury hospitalized dogs and evaluation of a veterinary acute kidney injury staging system. J Vet Emerg Crit Care 2011;21(6):648–657.
4. Savage VL, Marr CM, Bailey M, Smith S. Prevalence of acute kidney injury in a population of hospitalized horses. J Vet Intern Med. 2019;33(5):2294–2301.
5. Groover ES, Woolums AR, Cole DJ, LeRoy BE. Risk factors associated with renal insufficiency in horses with primary gastrointestinal disease: 26 cases (2000–2003). J Am Vet Med Assoc. 2006;228(4):572–577.
6. Von Hendy-Willson VE, Pressler BM. An overview of glomerular filtration rate testing in dogs and cats. Vet J. 2011;188(2):156–165.
7. Hall JA, Yerramilli M, Obare E, Yerramilli M, Melendez LD, Jewell DE. Relationship between lean body mass and serum renal biomarkers in healthy dogs. J Vet Intern Med. 2015;29(3):808–814.
8. Hokamp JA, Nabity MB. Renal biomarkers in domestic species. Vet Clin Pathol. 2016;45(1):28–56.
9. Toribio RE. Essentials of equine renal and urinary tract physiology. Vet Clin North Am Equine Pract. 2007;23(3):533–561.
10. Lo HC, Winter JC, Merle R, Gehlen H. Symmetric dimethylarginine and renal function analysis in horses with dehydration. Equine Vet J. 2021;00:1–9.
11. Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, et al. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol. (Oxf) 2013;207(4):663–672.

12. Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, et al. Neutrophil gelatinase–associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis. 2008;52(3):595–605.
13. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005;365(9466):1231–1238.
14. Cruz DN, de Cal M, Garzotto F, Perazella MA, Lentini P, Corradi V, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med. 2010;36(3):444–451.
15. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J, et al. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care. 2007;11(6):1–8.
16. Tuladhar SM, Püntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. J Cardiovasc Pharmacol. 2009;53(3):261–266.
17. Kai K, Yamaguchi T, Yoshimatsu Y, Kinoshita J, Teranishi M, Takasaki W. Neutrophil gelatinase-associated lipocalin, a sensitive urinary biomarker of acute kidney injury in dogs receiving gentamicin. J Toxicol Sci. 2013;38(2):269–277.
18. Davis J, Raisis AL, Cianciolo RE, Miller DW, Shiel RE, Nabity MB, et al. Urinary neutrophil gelatinase-associated lipocalin concentration changes after acute haemorrhage and colloid-mediated reperfusion in anaesthetized dogs. Vet Anaesth Analg. 2016;43(3):262–270.
19. Lee YJ, Hu YY, Lin YS, Chang CT, Lin FY, Wong ML, et al. Urine neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute canine kidney injury. BMC Vet Res. 2012;8(1):1–9.
20. Hansen S, Otten ND, Hopster-Iversen C, Fjeldborg J. *Neutrophil gelatinase-associated lipocalin (NGAL) as a potential biomarker for equine asthma* [dissertation]. Denmark, IL: Department of Veterinary Clinical Sciences, University of Copenhagen Faculty of Health and Medical Sciences.
21. Winther M, Haugaard S, Pihl TH, Jacobsen S. Neutrophil gelatinase-associated lipocalin in serum and peritoneal fluid from horses with abdominal disease. [Abstracts from the 13th International Equine Colic Research Symposium]. *BEVA Equine Veterinary Education*. 2021;33(special issue):16–17.
22. Frydendal C, Nielsen KB, Berg LC, van Galen G, Adler DM, Andreassen SM, et al. Influence of clinical and experimental intra-articular inflammation on neutrophil gelatinase-associated lipocalin concentrations in horses. Vet Surg. 2021;50(3):641–649.
23. Siwińska N, Żak A, Pasławska U. Evaluation of serum and urine neutrophil gelatinase-associated lipocalin and cystatin C as biomarkers of acute kidney injury in horses. J Vet Res. 2021;65(2):245–252.
24. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase–associated lipocalin. J Am Soc Nephrol. 2007;18(2):407–413.

25. Daure E, Belanger MC, Beauchamp G, Lapointe C. Elevation of neutrophil gelatinase-associated lipocalin (NGAL) in non-azotemic dogs with urinary tract infection. Res Vet Sci. 2013;95(3):1181–1185.
26. Schmidt-Ott KM. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury––where do we stand today? Nephrol Dial Transplant. 2011;26:762–764.
27. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol. 2003;14(10):2534–2543.
28. Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease. Scan J Clin Lab Invest, 2008;68(sup241):89–94.
29. Jacobsen S, Berg LC, Tvermose E, Laurberg MB, van Galen G. Validation of an ELISA for detection of neutrophil gelatinase-associated lipocalin (NGAL) in equine serum. Vet Clin Pathol. 2018;47:603–607.
30. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Group NMAI. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J of Kidney Dis. 2009;54(6):1012–1024.
31. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009;4(2):337–344.
32. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney Int. 2007;71(10):967–970.
33. Reed SM, Bayly WM, Sellon DC. Equine internal medicine, 4th edn. St. Louis, Missouri: Elsevier Inc.; 2018.
34. van Deursen VM, Damman K, Voors AA, van der Wal MH, Jaarsma T, van Veldhuisen DJ, et al. Prognostic value of plasma neutrophil gelatinase–associated lipocalin for mortality in patients with heart failure. Circ Heart Fail. 2014;7(1):35–42.
35. Cortellini S, Pelligand L, Syme H, Chang YM, Adamantos S. Neutrophil gelatinase-associated lipocalin in dogs with sepsis undergoing emergency laparotomy: a prospective case–control study. J Vet Intern Med. 2015;29(6):1595–1602.
36. Monari E, Troìa R, Magna L, Gruarin M, Grisetti C, Fernandez M, et al. Urine neutrophil gelatinase-associated lipocalin to diagnose and characterize acute kidney injury in dogs. J Vet Intern Med. 2020;34(1):176–185.
37. Thorsvik S, Bakke I, van Beelen Granlund A, Røyset ES, Damås JK, Østvik AE, et al. Expression of neutrophil gelatinase-associated lipocalin (NGAL) in the gut in Crohn's disease. Cell Tissue Res. 2018;374(2):339–348.
38. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med. 2008;36(4):1297–1303.

39. Geor RJ. Acute renal failure in horses. Vet Clin North Am Equine Pract. 2007;23(3):577–591.
40. Van der Harst MR, Bull S, Laffont CM, Klein WR. Gentamicin nephrotoxicity–a comparison of in vitro findings with in vivo experiments in equines. Vet Res Commun. 2005;29(3):247–261.
41. Pedersen KR, Ravn HB, Hjortdal VE, Nørregaard R, Povlsen JV. Neutrophil gelatinase-associated lipocalin (NGAL): validation of commercially available ELISA. Scand J Clin Lab Invest. 2010;70(5):374–382.
42. Chaney KP, Holcombe SJ, Schott HC 2nd, Barr BS. Spurious hypercreatininemia: 28 neonatal foals (2000^2008). J Vet Emerg Crit Care (San Antonio). 2010;20(2):244–249.
43. Roy MF, Kwong GPS, Lambert J, Massie S, Lockhart S. Prognostic value and development of a scoring system in horses with systemic inflammatory response syndrome. J Vet Intern Med. 2017;31(2):582–592.
44. Van Galen G, Jacobsen S, Vinther AM, Breinholt Laurberg M, Tvermose E, Broe-Brøndum R, et al. NGAL: a new biomarker in the horse for renal injury and inflammation. J Vet Intern Med. 2019;33:1549–1549.

Figures

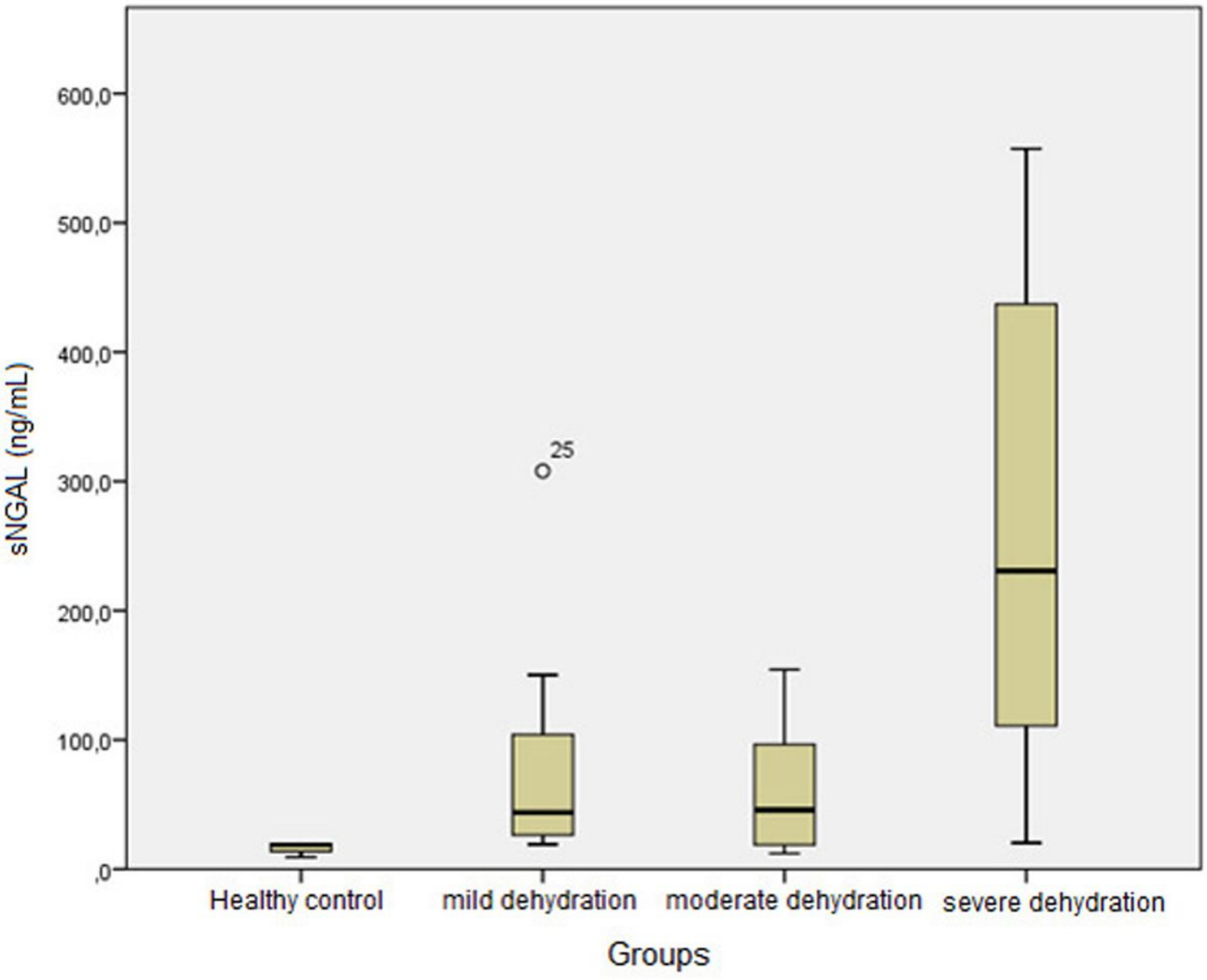

Figure 1

Distribution of serum neutrophil gelatinase associated lipocalin (sNGAL) concentrations at time-point 0 according to dehydration status. The sNGAL concentrations varied significantly among three dehydration groups (*P* = 0.02)

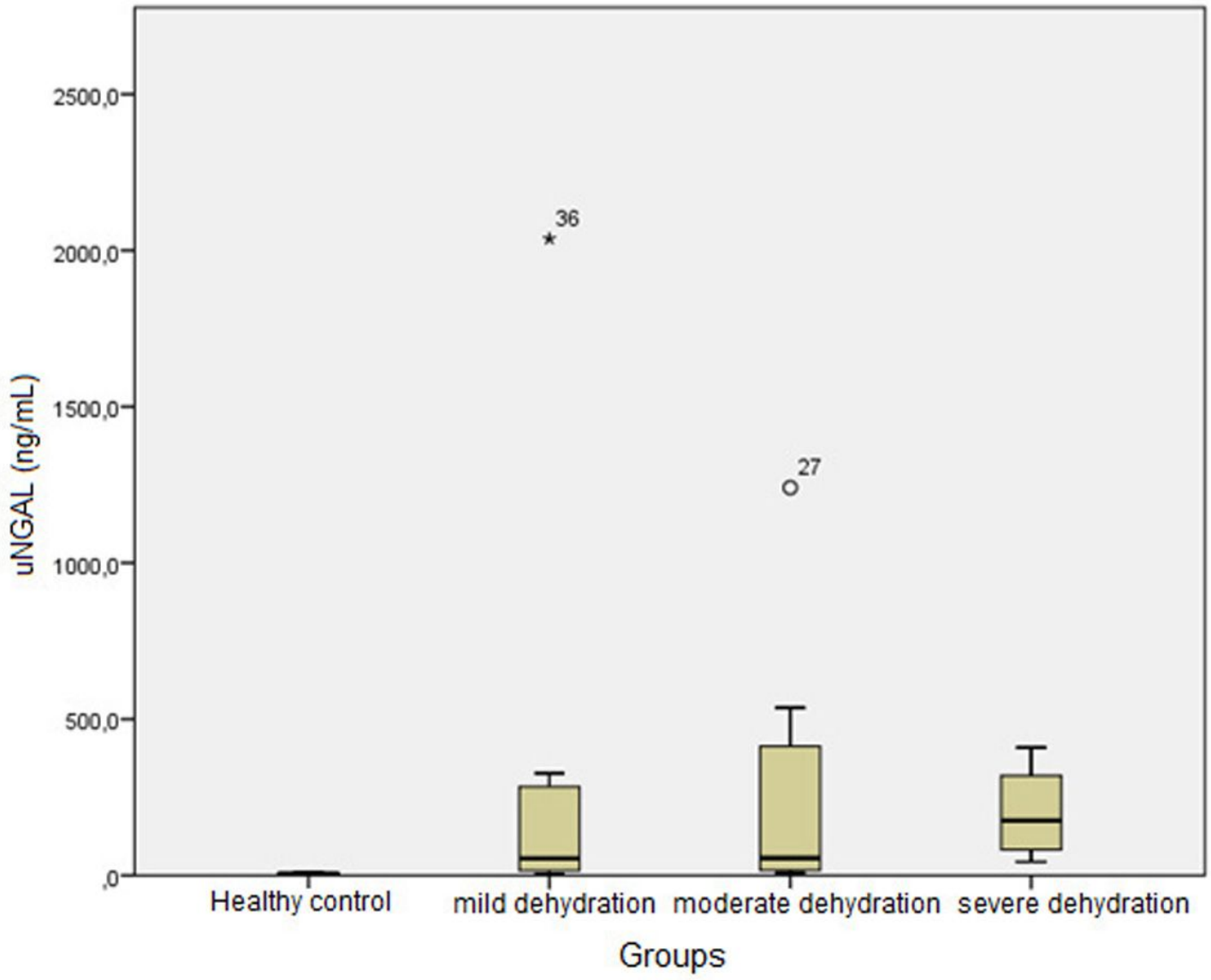

Figure 2

Distribution of urinary neutrophil gelatinase associated lipocalin (uNGAL) concentrations at time-point 0 according to dehydration status. The uNGAL concentrations varied significantly among three dehydration groups (*P* = 0.01)

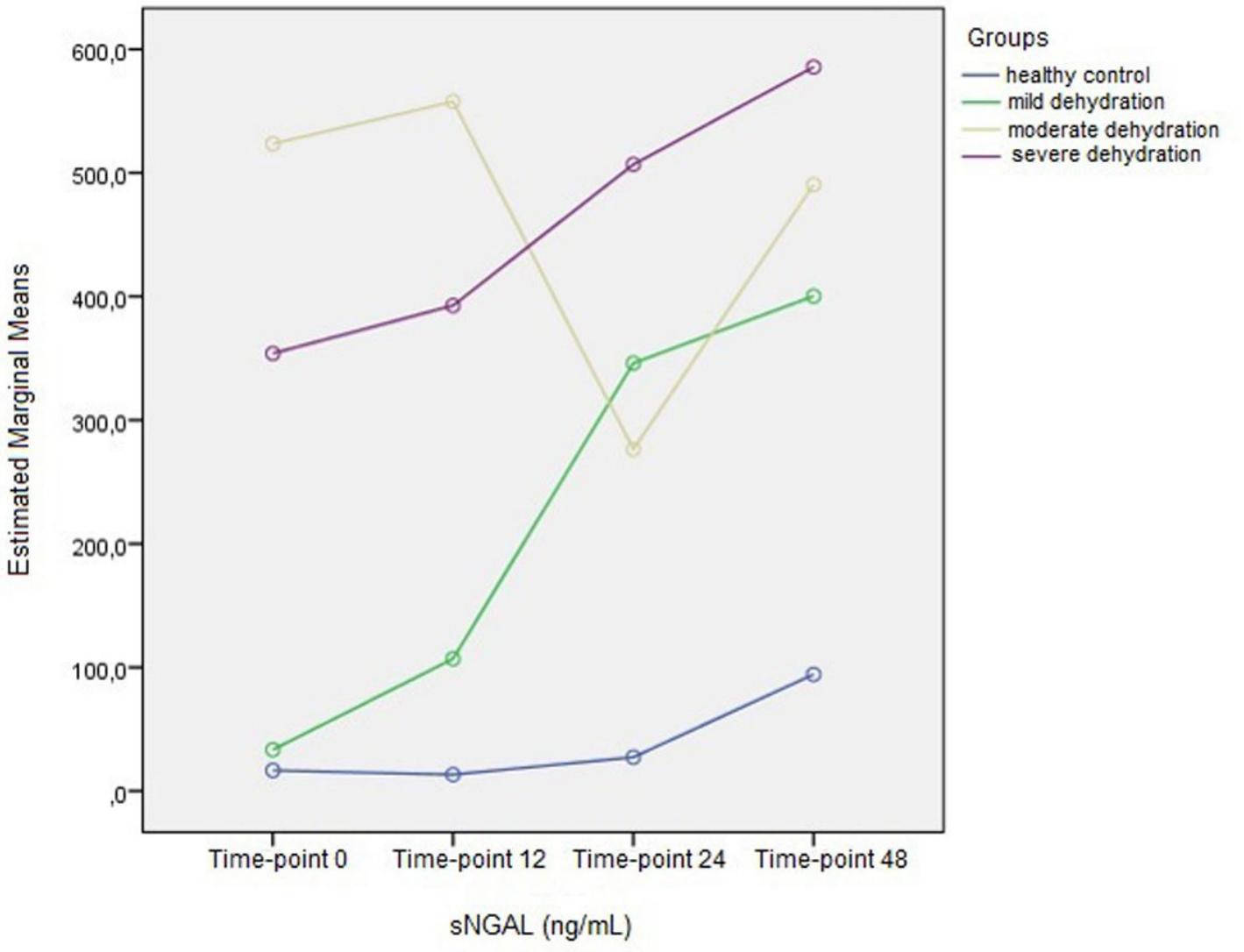

Figure 3

Estimated marginal means of serum neutrophil gelatinase associated lipocalin (sNGAL) in three different dehydration groups and healthy control horses within 48 h. The progression of sNGAL in 48 h in these four groups had no significant differences (P = 0.7).

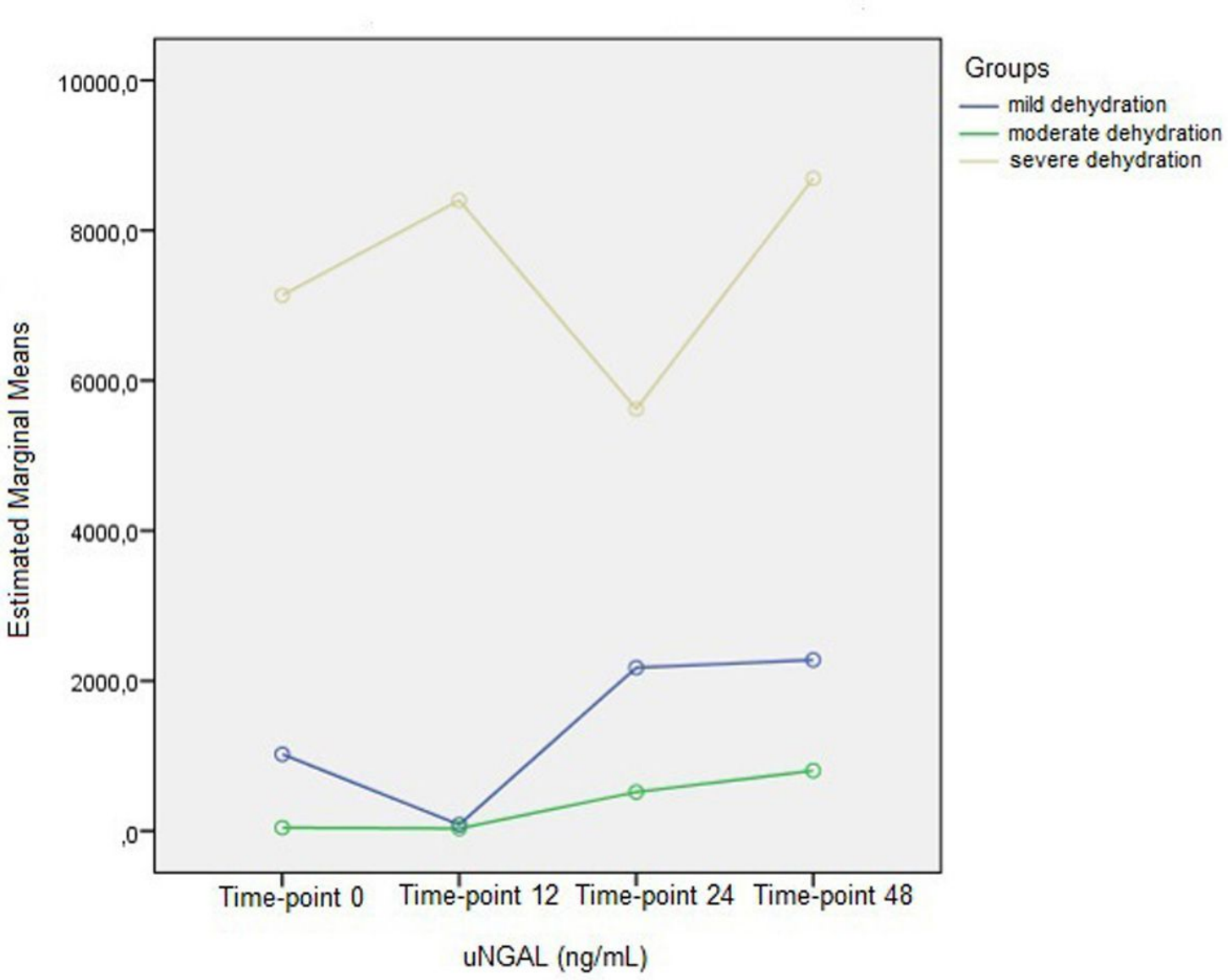

Figure 4

Estimated marginal means of urinary neutrophil gelatinase associated lipocalin (uNGAL) in three different dehydration groups and healthy control horses within 48 h. The progression of uNGAL in 48 h in these four groups had no significant differences ($P = 0.5$).

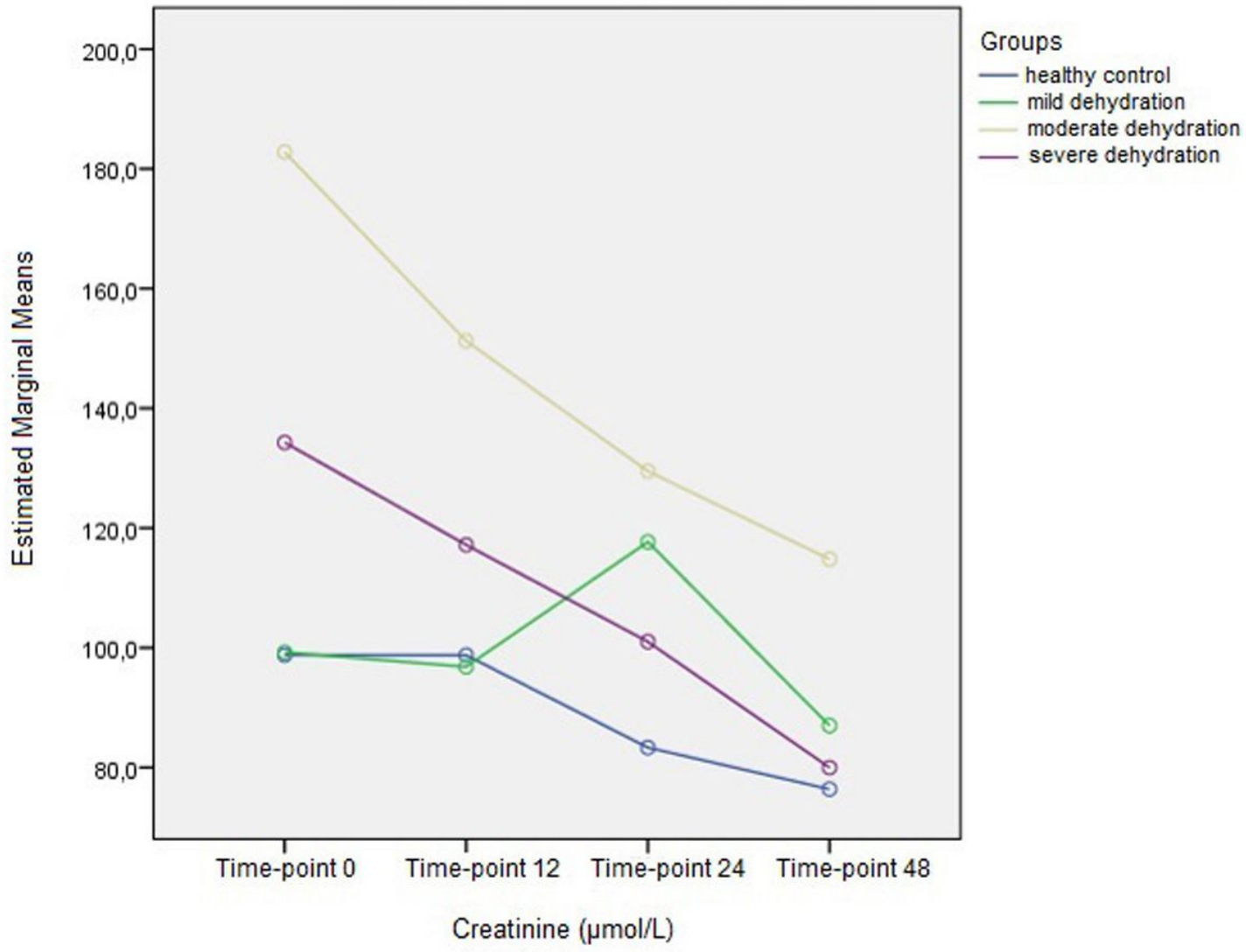

Figure 5

Estimated marginal means of serum creatinine in three different dehydration groups and healthy control horses within 48 h. The progression of serum creatinine in 48 h in these four groups had no significant differences ($P = 0.2$).

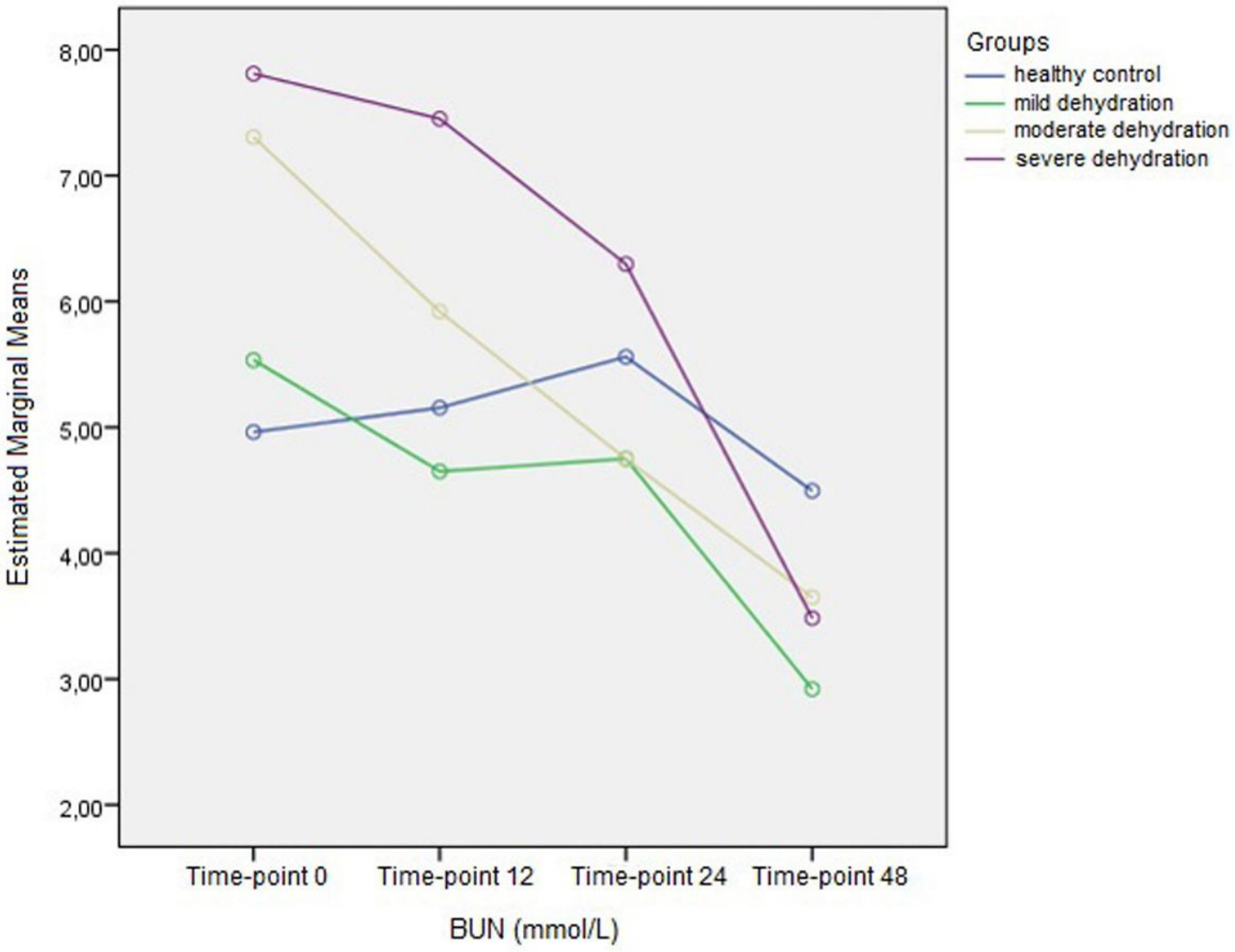

Figure 6

Estimated marginal means of blood urea nitrogen (BUN) in three different dehydration groups and healthy control horses within 48 h. The progression of BUN in 48 h in these four groups had no significant differences ($P = 0.4$).

Supplementary Files

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- Additionalfile1Parametersusedforestimationofdehydrationinthehorse.docx